EPA completed the most recent IRIS assessment of chloroprene in 2010. In that assessment, the agency concluded that chloroprene is "likely to be carcinogenic to humans" through a mutagenic mode of action and that the primary exposure route of concern is the inhalation pathway. Accordingly, the assessment included an inhalation unit risk (IUR), which is an estimate of inhaled cancer potency that can be used to estimate the risk of cancer that would be expected in a population exposed to chloroprene in the air every day over a lifetime.

Recently, the Office of Air and Radiation released the most recent version of the National Air Toxics Assessment, a national analysis that combines information about the emissions of specific air pollutants to estimate the risk of developing a particular health effect in a population. The most recent version of NATA was the first to incorporate information (i.e., the IUR) from the 2010 IRIS assessment for chloroprene, and it identified the census tract DuPont/Denka facility in La Place, LA (i.e. Lake Pontchartrain Works site) as having an elevated risk for cancer.

In response to this designation, scientists from Ramboll Environ, as representatives of DuPont/Denka briefed Agency scientists on specific issues related to the chloroprene assessment and new studies published since the release of the 2010 IRIS assessment. The conclusion of the Ramboll Environ scientists was that the new analyses warranted a sufficient reason for IRIS to re-evaluate the science surrounding chloroprene and to update the IRIS assessment and derive new risk values.

The purpose of this memo is to provide information on EPA's evaluation of the three recent studies identified by Ramboll Environ scientists, as well as issues raised to Agency staff by Ramboll Environ scientists during their briefing on August 9th, 2016. Specifically, Ramboll Environ scientists raised the following issues: 1) toxicological evidence of carcinogenicity and mode of action; 2) possible toxicokinetic differences between mice and humans; and 3) combined dose-response analysis of epidemiological and toxicological data.

Possible inter-species differences in toxicokinetics and PBPK modeling

Yang et al. (2012) presents the results of the refinement of an existing deterministic physiologically-based pharmacokinetic (PBPK) model and the development of a new probabilistic PBPK model. Upon review, there are many apparent concerns about the results presented in this study.

EPA staff does not currently have access to all data and model codes from the Yang et al. (2012) publication, which would be necessary for a thorough quality assurance review. However, based upon the materials available in Yang et al. (2012) and comments submitted to Docket ID: EPA-HQ-ORD-2009-0217, the model is of inadequate quality for use in an IRIS dose-response assessment without significant revisions. As a result, the impact of the toxicokinetic studies on dose-response analysis and rodent-to-human extrapolation is unknown.

Female mouse lung metabolism and internal doses in Yang et al. (2012) are not consistent with

results for male mice, or values previously calculated by Himmelstein et al. (2004a). Tables 3 and 4 of Yang et al. (2012) report lung Vmax to be approximately 5 times higher for male mice than for female mice. Not surprisingly, the male mouse internal lung dose metric is over 5-fold higher than the female mouse at each exposure concentration (Table 5 of Yang et al., 2012). However, the tumor profiles between male and female mice are very similar: 26% and 8% (control); 56% and 57% (12.8 ppm); 72% and 68% (32 ppm); and 86% and 84% (80 ppm) (NTP, 1998). Since the fundamental premise of this series of papers is that mouse lung tumors may not be relevant to humans given the large differences in lung metabolism, the reported differences in the internal dose metrics between male mice and female mice should have elicited explanation by the authors. If tumor response can be better explained by using internal dose vs. external concentration, it is unclear how such large differences in metabolism do not translate to differences in tumor incidence. The difference of internal dose between male and female mice is similar to the difference between female mice and humans (Table 5 of Yang et al., 2012): 7.4 (at 12.8 ppm), 4.8 (at 32 ppm), and 2.5 (at 80 ppm). The subsequent dose-response analysis by Allen et al. (2014) only incorporates female mouse data, and no rationale for the omission of male mouse data are provided.

Female mouse liver and kidney metabolism may be underestimated in Yang et al. (2012). For liver metabolism, this is apparent on the log-scale for predictions of chloroprene headspace concentration data provided in Figure 2b of Yang et al. (2012), and Figures 5 and 25 of Study IISRP-17520-1388 (submitted to EPA-HQ-ORD-2009-0217). The underestimation occurs for both the point estimate results and the Monte Carlo results. For kidney metabolism, anomalies are apparent in the output distributions of the metabolic parameters Vmax and Km for female mice [Figure S6 of Yang et al. (2012) supplementary materials, and Figure 20 of Study IISRP-17520-1388]. Unlike for male mice, the probability samples cluster around zero for female mice. The underestimation only occurs for the Monte Carlo results, and the difference between point estimates and Monte Carlo estimates (which are a factor of 10 lower) is attributed only to "background loss rate". It is possible that there was an error in the Markov-Chain Monte-Carlo (MCMC) optimization, and that kidney metabolism is greatly underpredicted in female mice.

The metabolic data used to parameterize both the deterministic and probabilistic PBPK models were generated via in vitro headspace experiments where chloroprene was added to closed vials with lung, liver, or kidney microsomal preparations and the disappearance of chloroprene from the vial headspace was measured. Microsomes are derived from the endoplasmic reticulum that contain Phase I and II metabolizing enzymes; microsomes are not present in living cells and are not capable of transcribing mRNA. Thomas et al. (2012) points out that induction of metabolizing enzymes appears to differ between rats and mice. Cyp2e1 mRNA levels in rats (exposed to 200 ppm chloroprene for either 5 or 15 days) were increased over controls but no increases were seen in mice. Conversely, epoxide hydrolase mRNA was induced in mice at > 13 ppm (5 or 15 days) and > 3 ppm (5 days only), but not rats. Thomas et al. (2012) states "It is not yet known whether the changes in Cyp2e1 and Ephx1 mRNAs are translated into increased enzyme activity, but the ultimate result would be a narrowing of the cross-species differences in the activation-to-detoxification ranges."

This is a very important point as, since the PBPK model is built on *in vitro* data that used microsomal fractions, this data would not capture any upregulation of activation and detoxification enzymes. Evidence of enzyme induction in vivo that is not captured in a PBPK model built on *in vitro* data would limit the model's validity. The lack of Cy2e1 induction in mice is supported by an unpublished report submitted to the chloroprene docket (EPA-HQ-ORD-2009-0217-0009, report IISRP-12828-1406). This report states that, "after 15 days of inhalation exposure to β-Chloroprene, no dose-dependent alterations were observed in total CYP content or CYP 1A2, 2B1/2, 2E1, 3A2 or 4A1/2/3 content."

Also on the chloroprene docket, is a report in which blood chloroprene was measured in mice following single (6 hour) and repeated (5 day or 15 day) inhalation exposures. Chloroprene blood levels were higher following single exposures, which was postulated to be because of higher minute volume due to stress. The authors conclude that this blood data is suitable for validation of a PBPK model, but it is unclear whether the data was used for the validation of the PBPK model in Yang et al. (2012). The report did not investigate chloroprene levels in the organs of interest (namely the lungs, liver, or kidneys).

Toxicological evidence of carcinogenicity and mode of action

In their comments on the chloroprene assessment, Ramboll Environ scientists questioned the scientific support for a genotoxic mode of action for chloroprene, and instead proposed an alternative mode of action involving hyperplasia, induced cell proliferation, and increased expression of pre-existing mutations. The 2010 assessment does not discount the possibility of additional carcinogenic modes of action, and even acknowledges that alternative modes of action may be present at high doses given the decrease in K-ras A to T transversions seen at high doses (i.e., 80 ppm). However, the evidence presented in the 2010 IRIS assessment clearly supports that genotoxicity is a possible mode of action. Ramboll Environ scientists not that A to T transversions have been observed in spontaneous mouse lung tumors, but this particular transversion (CAA → CTA at codon 61) was not observed in any historical NTP controls, thus decreasing the chance that chloroprene exposure could be increasing the expression of pre-existing mutations. Further, the proposed genotoxic mode of action for chloroprene was unanimously supported by the external peer review committee that reviewed the assessment.

Also, interestingly, most of the studies on which Ramboll Environ scientists cite to support their proposed application of the PBPK model also conclude or report that chloroprene may operative via a mutagenic mode of action. For example, the three Himmelstein toxicokinetic papers all make statements in their introductions regarding the mutagenicity of chloroprene. Himmelstein 2001b and 2004a state that in some tests, but not others, chloroprene appears to be genotoxic. Himmelstein 2004b states more strongly that "[t]he mechanistic steps by which CD exposure leads to rodent tumors, while not understood fully, strongly suggest a genotoxic mode of action. Himmelstein 2001a tested the mutagenicity and clastogenicity of (1-chloroethenyl)oxirane and concluded that "results suggested that CEO-induced mutagenicity, but not clastogenicity, may contributed to CD-induced carcinogenicity." The three papers under current consideration (Yang et al., 2012; Thomas et al., 2012; Allen et al., 2014) also make strong statements regarding chloroprene's mutagenicity:

- Thomas (2012) – "[t]he current hypothesized mode of action for chloroprene involves bioactivation to a mutagenic metabolite, leading to DNA damage and increased tumors."
- Yang (2012) – "[o]ne reactive intermediate formed is the epoxide (1-chloroethenyl)oxirane

which was mutagenic in the Ames assay, but not clastogenic at cytotoxic concentrations in vivo. This epoxide also shows reactivity with DNA in vitro and is a potential cross-linking agent."

- Allen (2014) – "[t]he initial step in metabolism is oxidation forming a stable epoxide, (1-chloroethenyl)oxirane, a genotoxicant that might be involved in the observed carcinogenicity in animals."

Toxicogenomics of chloroprene exposure

The Thomas et al., (2012) study conducted a transcriptomic dose-response analysis for the purpose of identifying possible modes of action to explain differences in cross-species tumor rates between mice and rats. However, while the limitation of the exposure durations to 5 and 15 days may be useful for identification of affected gene pathways, it remains unclear how these up or down regulations in gene expression relate to possible modes of action of the effects due to chronic exposures to chloroprene as addressed in the 2010 assessment.

Also notably missing from the analysis is any data on humans. While characterizing possible explanations for inter-species differences seen between mice and rats, characterizing differences between mice and humans would have been more informative.

Dose-response of human and animal toxicity data

The methodology of Allen et al (2014) has potential for reconciling dose-response relationships from humans and animals when it is not feasible to consider both data types on compatible dose and response scales. However, the reported chloroprene analysis did not use the hazard identification conclusions and dose-response approaches that the 2010 IRIS assessment relied on, so not surprisingly it estimated a different inhalation unit risk for respiratory cancer than the IRIS assessment. In addition, the use of the PBK metrics of Yang et al. (2012) for both humans and mice as critical inputs had an unclear impact, owing to the unexplained different rates of chloroprene metabolism in the lung between female and male mice and the unknown impact on projected human internal dose.

The primary difference concerns the human response data for respiratory cancer. The Allen et al. (2014) analysis was based solely on the standardized mortality ratios (SMRs) with external comparison (using US respiratory cancer rates) from the epidemiological study by Marsh et al (2007). In general, analyses based on internal controls are considered more valid and relevant given concerns including biases such as the healthy worker and healthy worker survivor effects. Therefore, these SMRs represent biased estimates, so the slope of zero for the Louisville cohort likely underestimated the magnitude of human responses.

Although there was insufficient support for dose-response estimation, EPA concluded in the 2010 assessment that there was an association of respiratory cancer with increasing chloroprene exposure. The most compelling evidence in the Marsh et al (2007) paper were the consistent associations, using internal controls, in every upper cumulative exposure quartiles (3 and 4) in the other 3 plants (odds ratio (OR) range: 1.9-2.9), as well as ORs in excess of 1.0 for low-level exposures in 2 out of 3 plants for quartile 2. Additionally, the cumulative exposure for the Louisville referent group (< 4.747 ppm*year) overlapped the exposures in 2nd quartile for the Maydown plant and the 2nd and 3rd quartiles for the Pontchartrain and Grenoble plants. EPA's interpretation of the human evidence was supported by the external peer review panel. Thus the choice of the Louisville cohort alone for the Allen et al. (2014)

analysis is curious. Thus, given the associations seen in the Maydown, Pontchartrain, and Grenoble cohorts among participants with low exposure levels, if there are low-level associations in the Louisville cohort, the referent choice would attenuate all associations in higher exposure levels compared to it. This would lead to an underestimated slope for the association between chloroprene exposure and lung cancer in that cohort. Thus lead to an underestimate of the IUR using the approach of Allen et al. (2014) when combining animal and human data.

Another difference in hazard identification conclusions between the Allen et al. (2014) and the 2010 IRIS assessment concerns multiple tumors observed in mice (and rats), and less sufficient evidence in humans to rule out this possibility. Concerning dose-response approaches, Allen et al. (2014) used a dose-response model that ignored data for decreased time to death with tumor in the mice. Although the human evidence did not support a model including this factor, earlier appearance of tumors was noted in several human studies. Both considerations contributed to a lower potency estimate in mice in the Allen et al. (2014) analysis.

Allen et al. (2014) omitted key information that would clarify applicability of the analysis. First, additional specifics of the dose-response point that both models were constrained to fit would have facilitated a better understanding of the analysis. That is, the cumulative human exposure (either in ppm-years or µmole of metabolite/g lung/day*years) corresponding to the daily PBK dose of 0.00352 µmole of metabolite/g lung/day was not provided, nor was the response (or range of responses in the uncertainty analysis) estimated at that exposure point.

A second point of needed clarification concerns the final ~1000-fold range of slope factors, which apparently reflects an uncertainty analysis that only considered the impact of assignments of chloroprene exposures in the Louisville cohort. Without information to clarify what was done, the "maximum-likelihood estimate" within this range then appears to be the slope factor estimate associated with the highest maximum-likelihood combined model fit among all maximum-likelihood estimates from 1500 different characterizations of the Louisville exposure data. Therefore, both limits of this range, as well as the central tendency estimate, are likely underestimated by considering only dose-response inputs that minimize estimates of human and animal potency, as opposed to considering the full range of interpretations consistent with the available data. Note: The EPA inhalation unit risk is an upper bound, and not directly comparable to a maximum-likelihood estimate.

General Conclusions

The three studies evaluated above represent novel approaches to analyzing existing epidemiologic, toxicological, and toxicokinetic data available for chloroprene. However, as is evident in the discussions of those studies, it is the opinion of the EPA that these studies do not present sufficient evidence or provide adequate rationale for re-evaluating the entire chloroprene toxicity database or deriving new reference values and/or cancer potency values. Of particular note, there are a number of serious concerns surrounding the development and/or application of the PBPK models (Yang et al. 2012), including poor model optimization of the derived metabolic parameters, and evidence of enzyme induction in exposed mice and rats that is not currently accounted for in the PBPK model. Thomas et al. (2012) provides only information on gene expression resulting from acute exposures, and likely does not reflect changes in gene expression or modes of action due to chronic exposure, limiting its utility in a chronic human health assessment. Last, the combined dose-response analysis (Allen et al., 2014) relied on judgments that underestimated risk in female mice and particularly underestimated human risk,

given existing data. Collectively, the new analyses do not support the conclusions that human risk of respiratory cancer is up to 100-fold less than that in female mice.

Ultimately, the Agency stands behind the conclusions made in the 2010 IRIS Toxicological Review of Chloroprene, including the derived cancer values. The new studies on chloroprene do not provide a reasonable basis for reassessing the human health effects due to chronic chloroprene exposure.

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